

研究例 2

未分化細胞の 分化制御

Extracellular syntaxin4 impacts on cell adhesion and differentiation of embryonal carcinoma F9 cells.

細胞外シンタキシンによるEC細胞の接着と分化への影響

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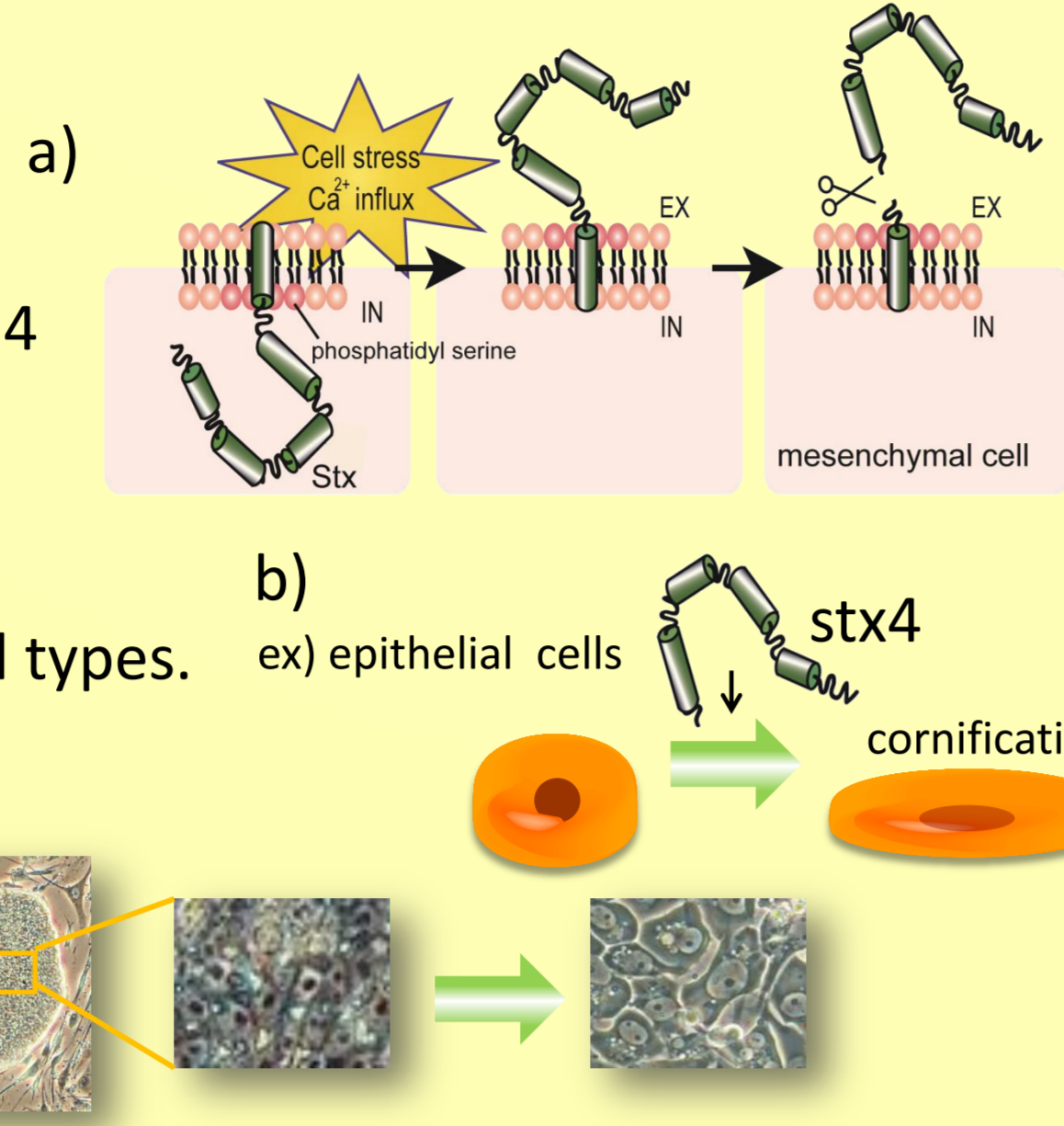
Background and our question

syntaxis (stxs)

- a) Subpopulations of certain syntaxis including syntaxis4 translocate across the cell membrane in response to external stimuli.
- b) The extracellularly presented syntaxis regulate morphology and differentiation of several epithelial cell types.

Anaplastic cells

- c) The differentiation is accompanied by the cell shape change.

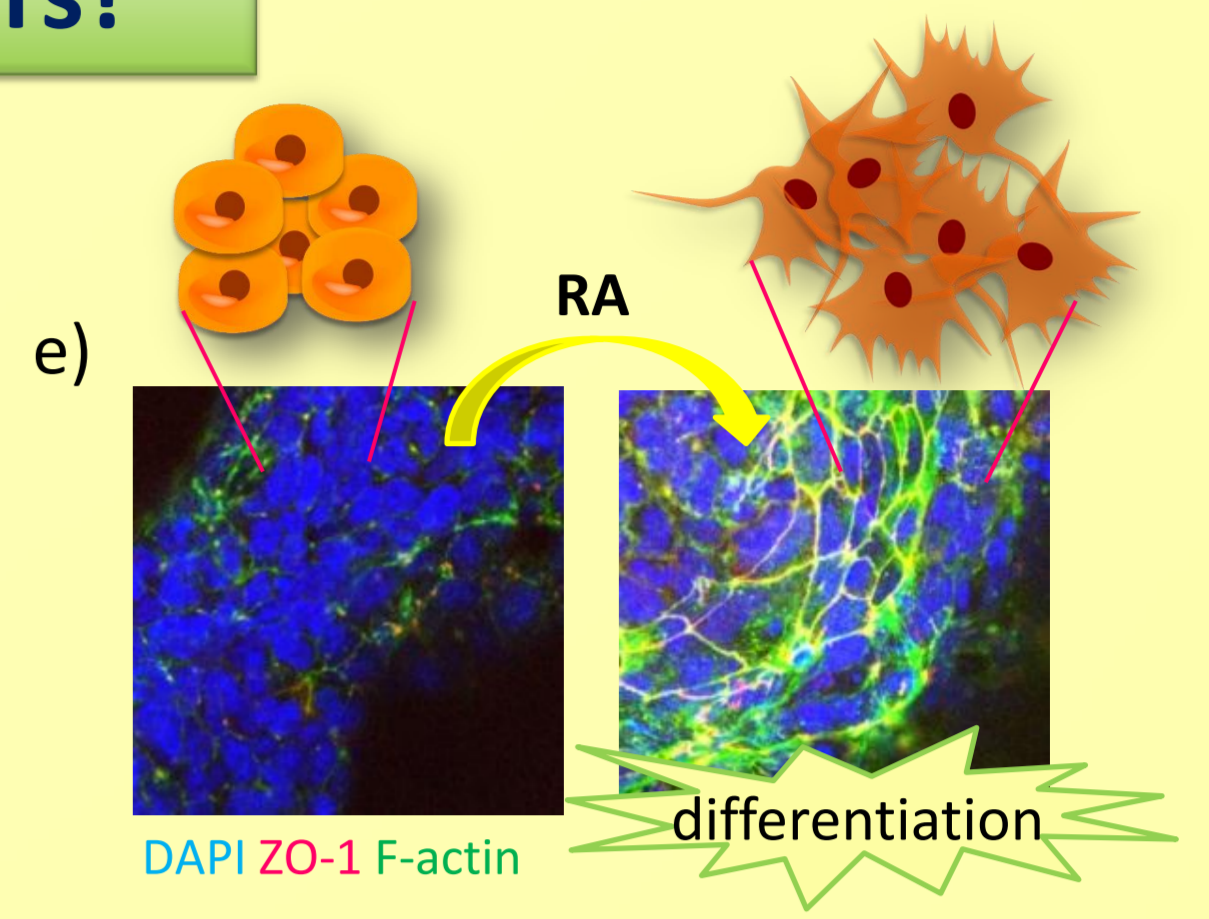


<OUR QUESTION>

Extracellularly presented syntaxin4 affects anaplastic cell behaviors?

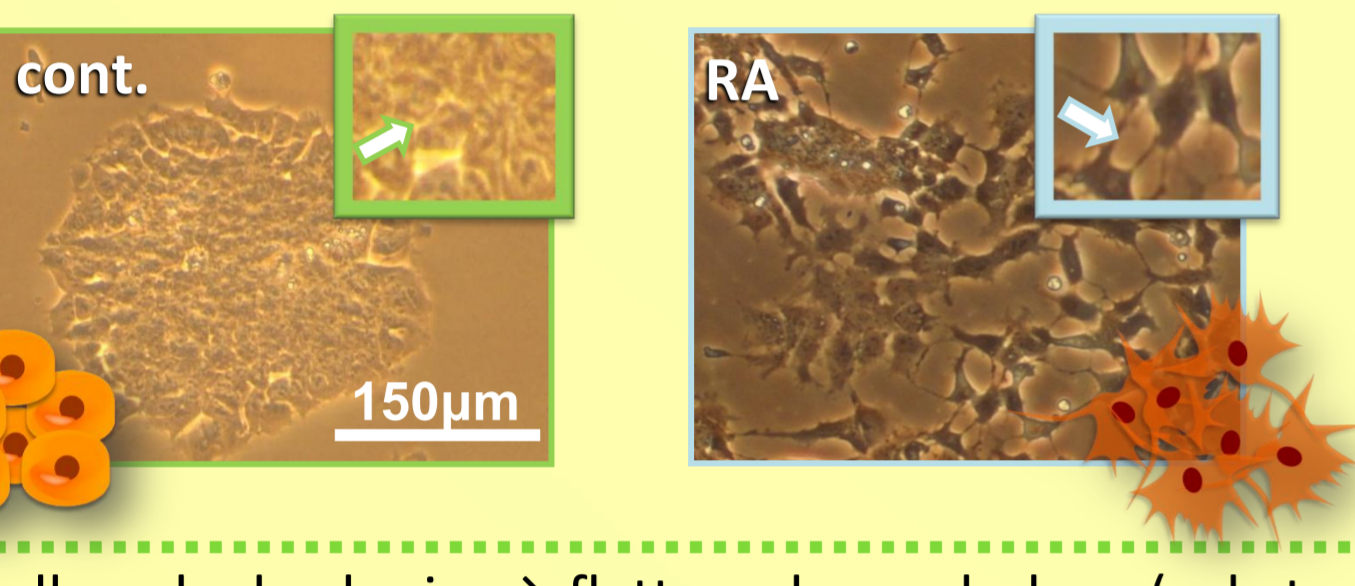
Model: F9 cells

- d) Mouse teratocarcinoma
- e) The method for the unidirectional differentiation to the endodermal lineage has been well established with using all-trans retinoic acid (RA).

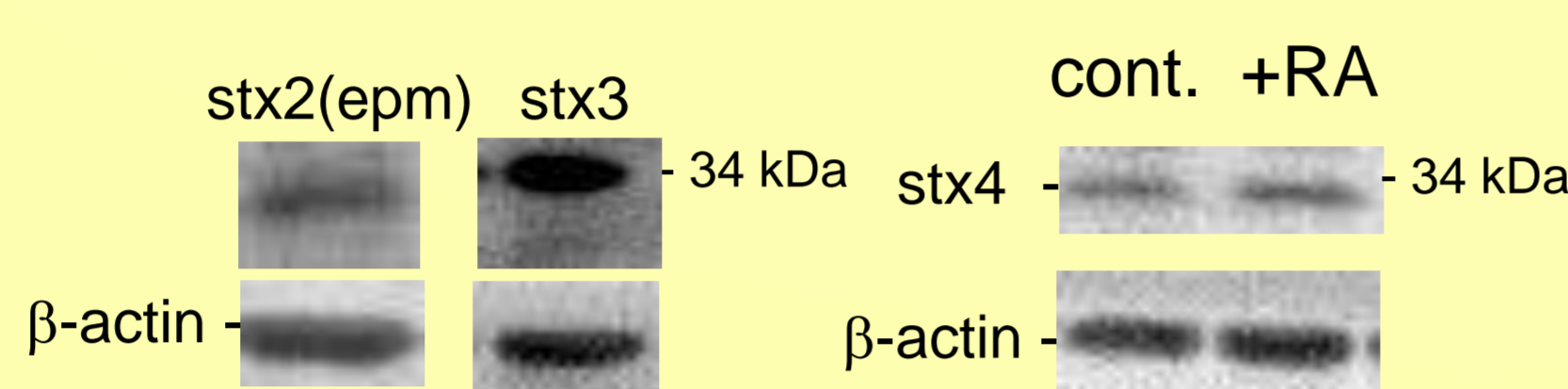


① Morphology in RA treated F9 cells and the surface expression of syntaxin4

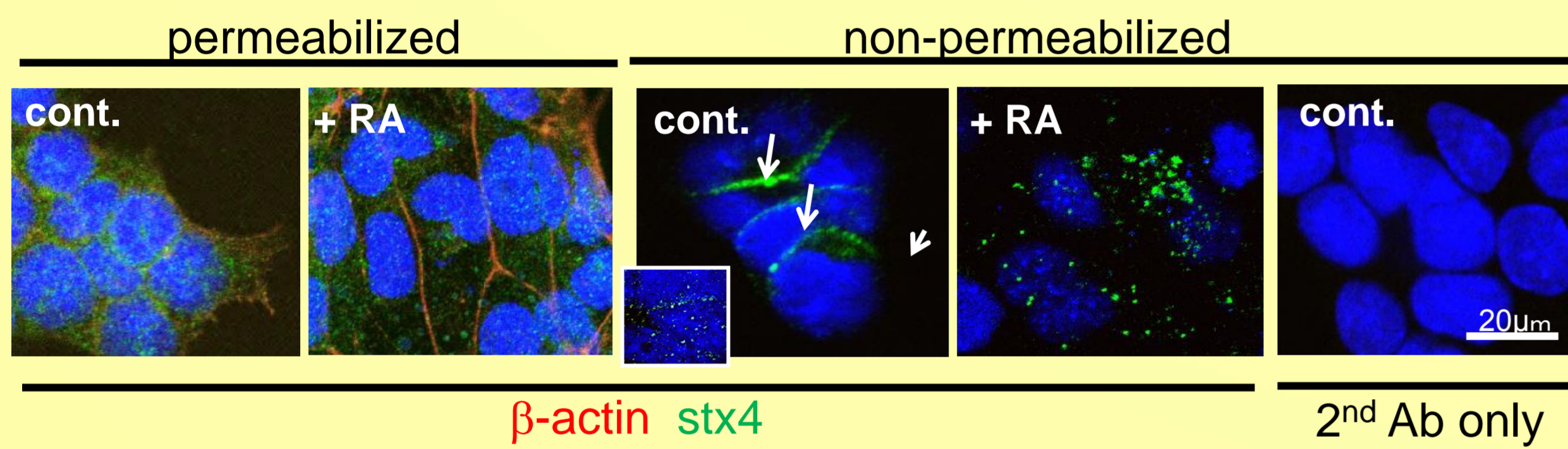
morphology



endogenous



Amount of stx4 was not affected by differentiation induction.



F9 cells
Extracellular subpopulation of stx4 localized at the lateral membrane.

Differentiation induction perturbed stx4's polar localization.

② Effect of stx4 on morphology

recombinant stx4 → F9 → cell shape change → flattened morphology (≅ RA)

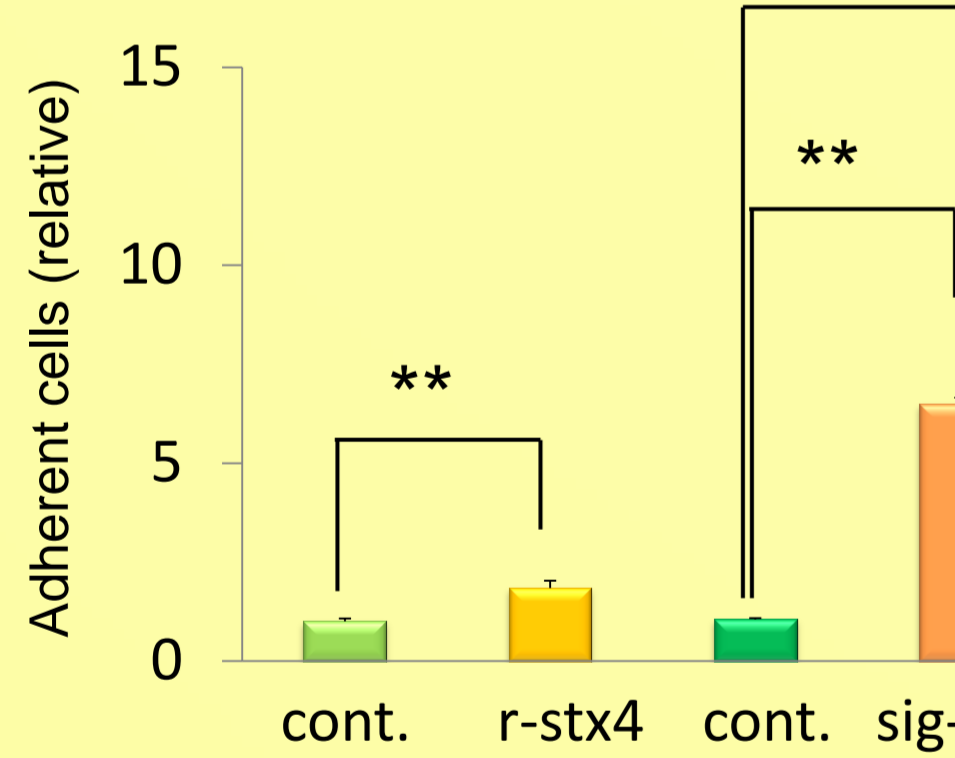
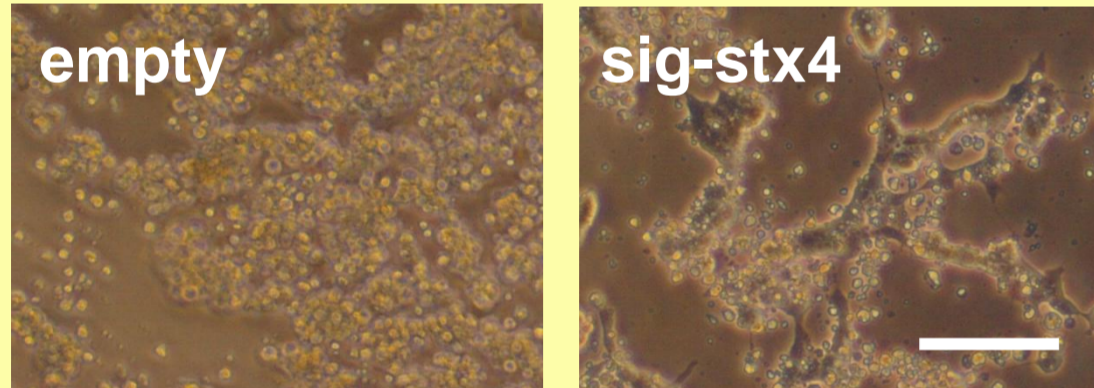
parent sig-stx4 (T7) → 34kDa, beta-actin

Colonies → Flattened morphology

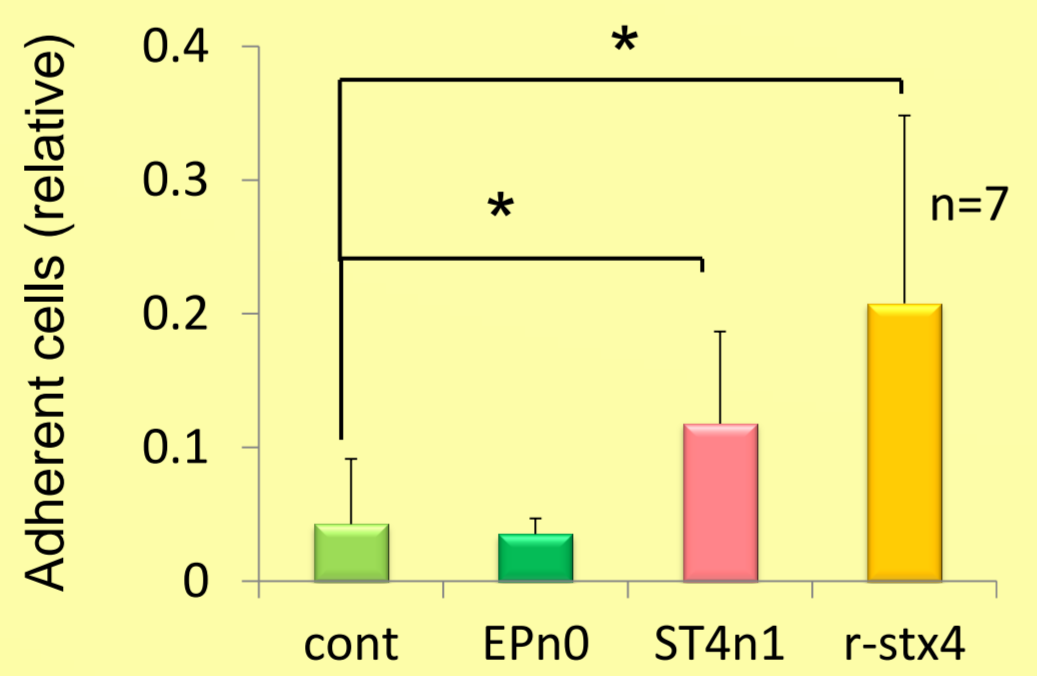
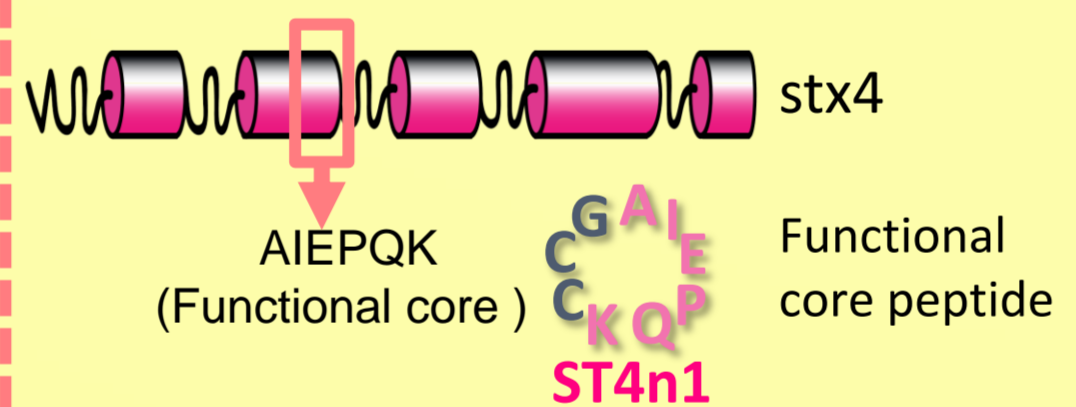
Extracellularly stx4 led to flattened and spreading morphology (≅ RA).

③ Effect of stx4 on cell-substrate adhesion

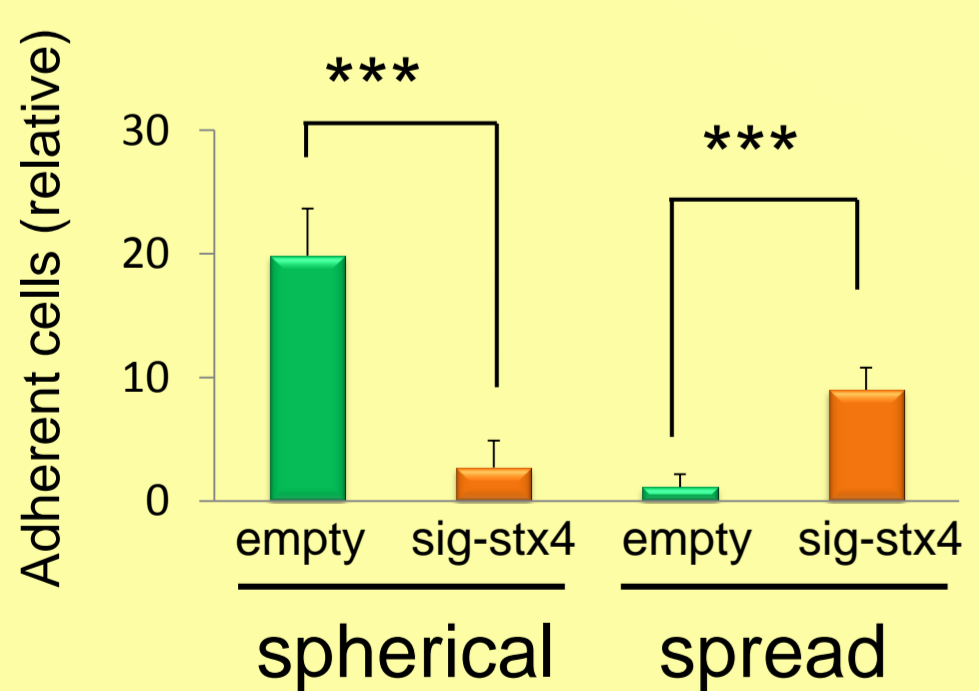
Long term adhesion (starved)



Functional core peptide

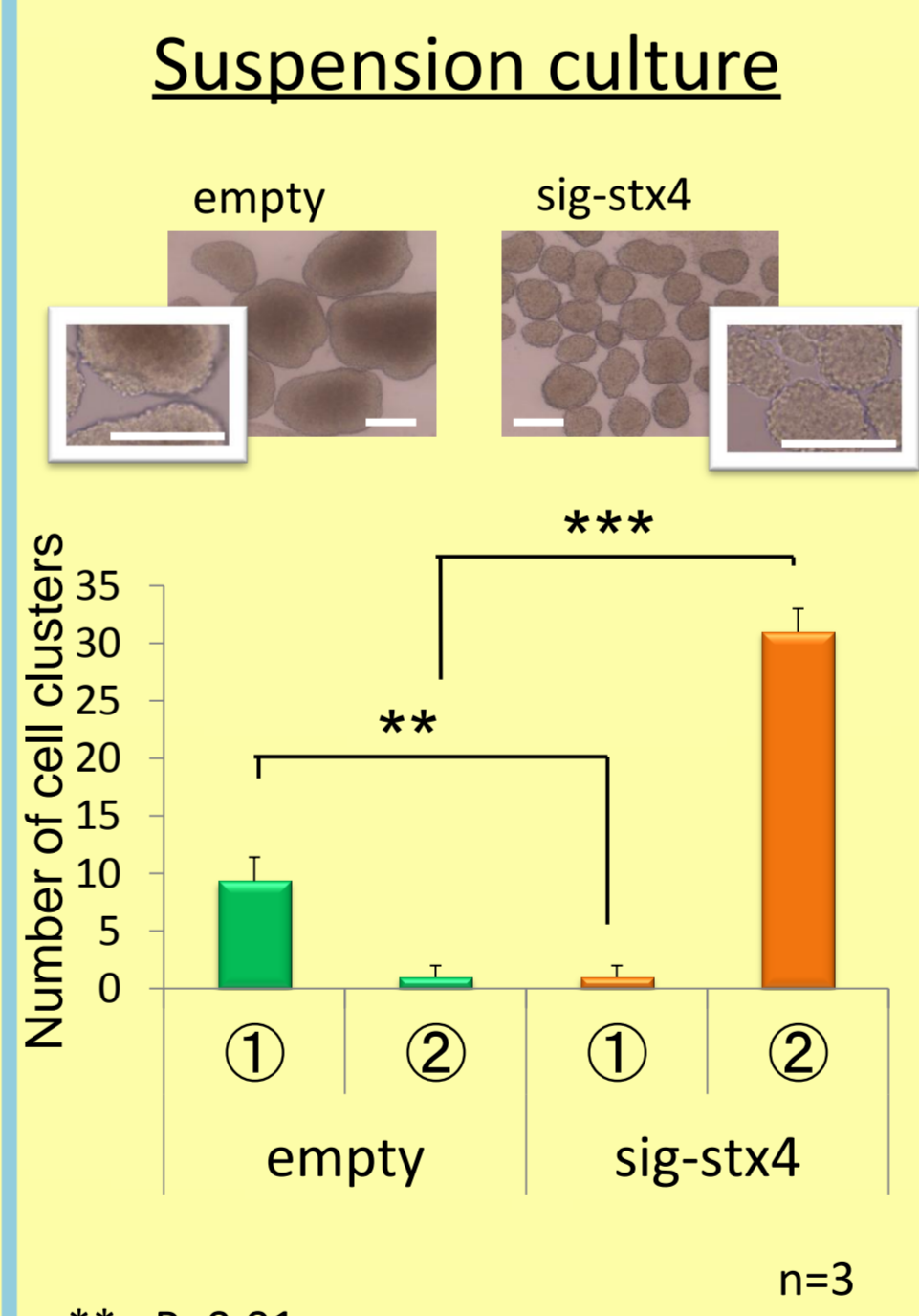
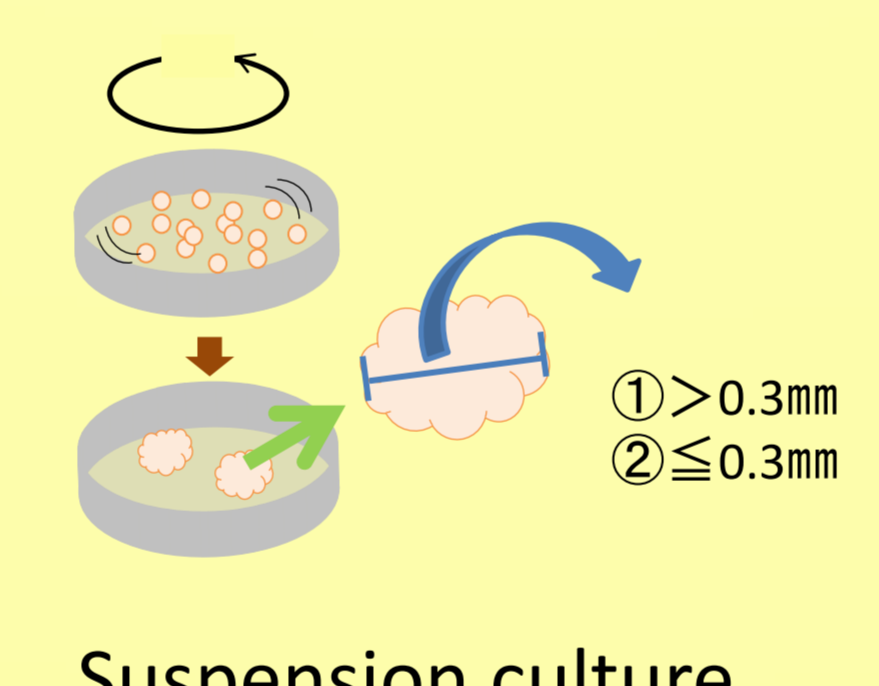


Adhesion / Spreading onto petri dish



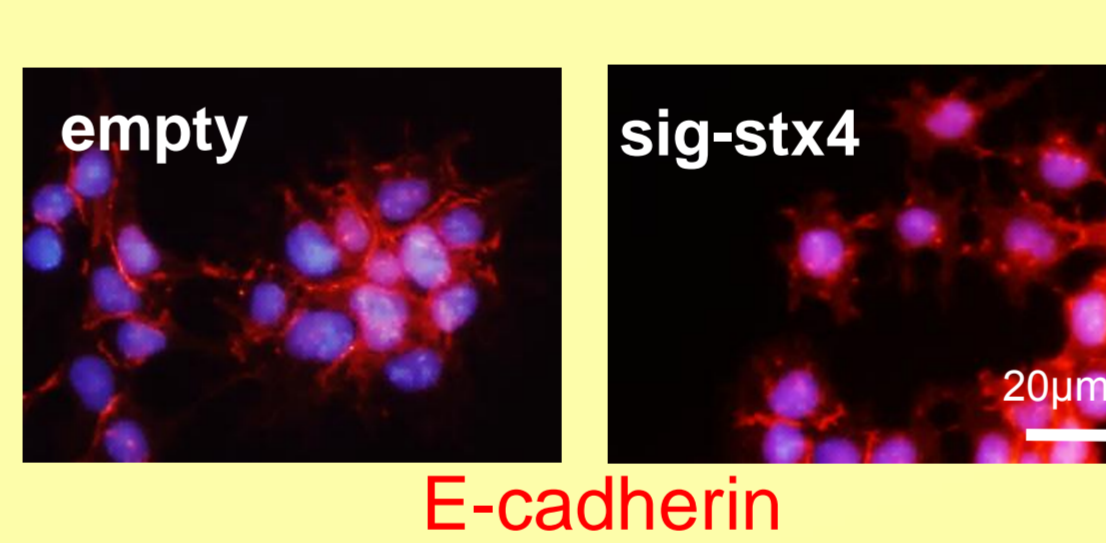
Extracellularly supplied Stx4 enhance cell-substrate adhesion

④ Effect of stx4 on cell-cell adhesion

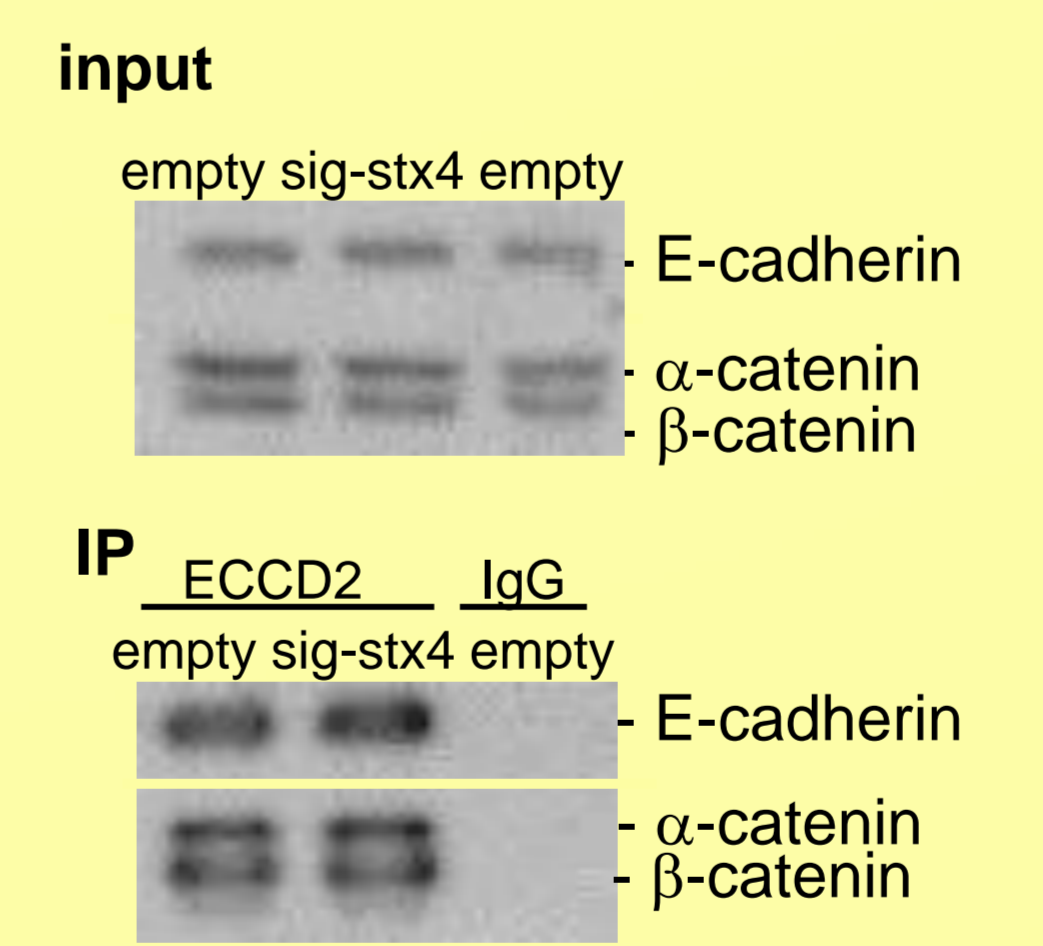


Stx4 → cell-cell adhesion ↓
< Cadherin-Catenin complex was not affected >

Localization of E-cadherin

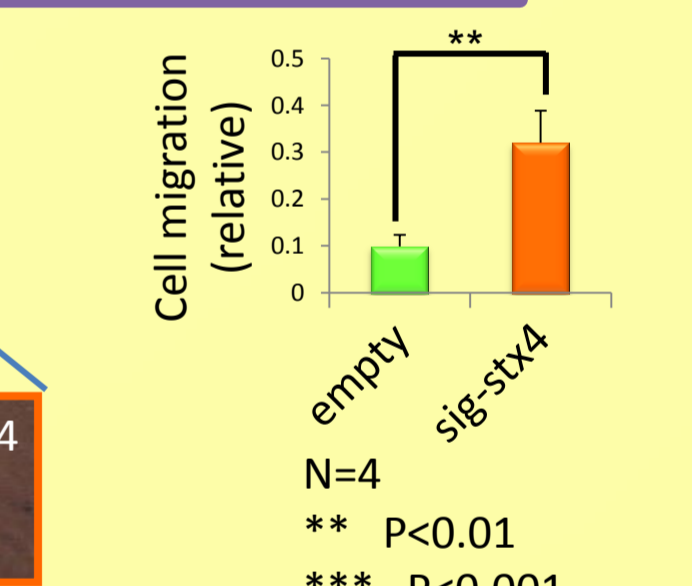
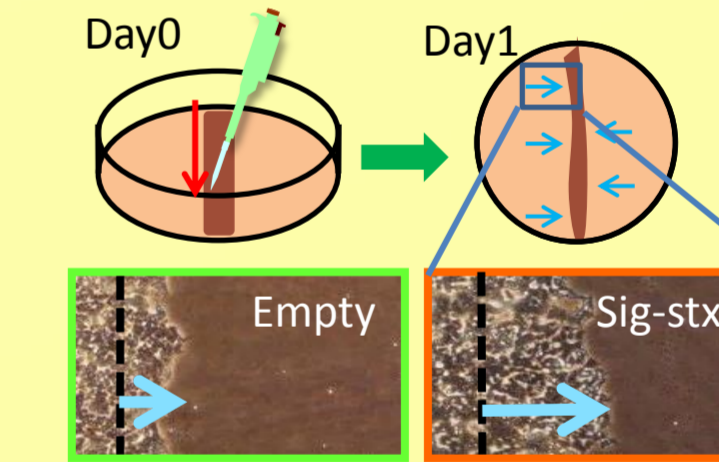


E-cadherin-Catenin complex

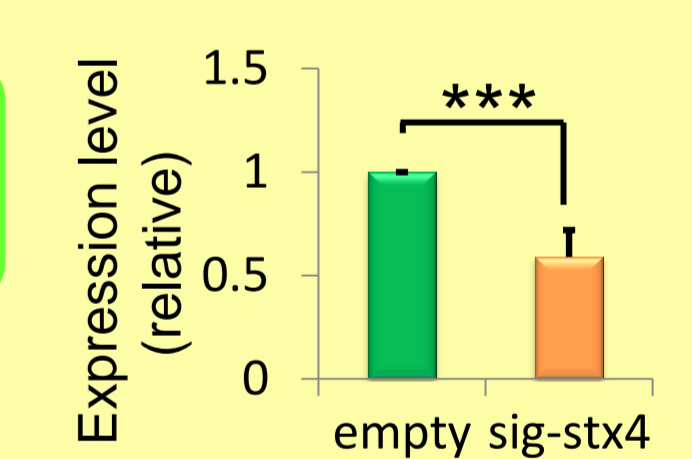
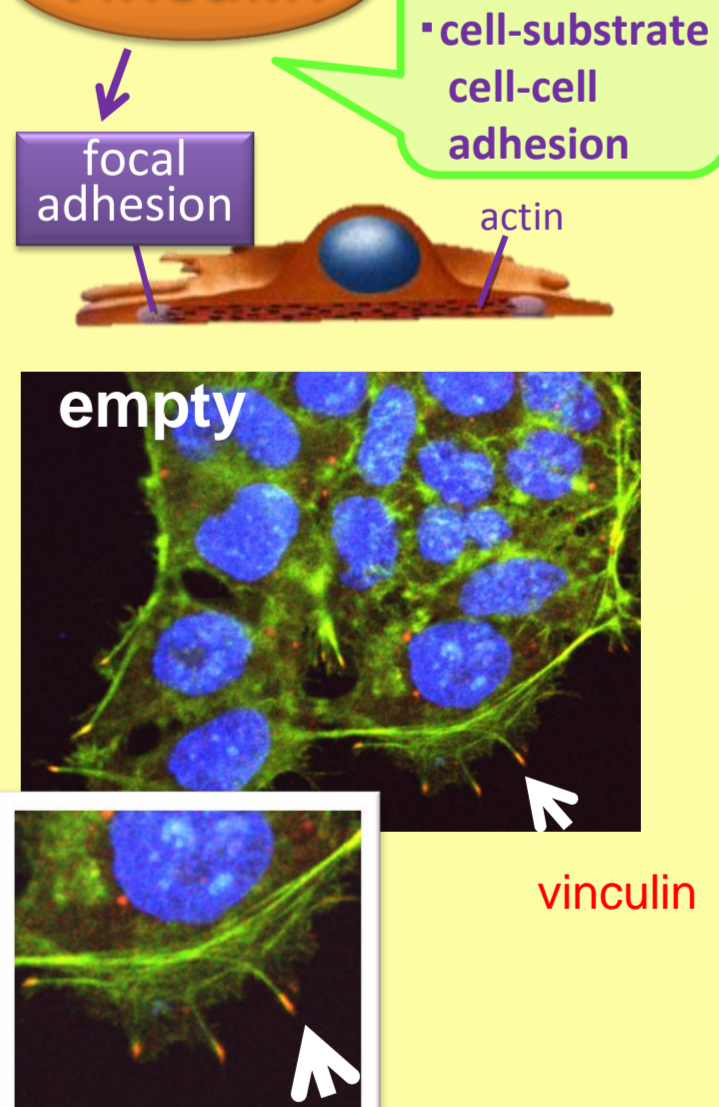


⑤ Effect of stx4 on migration and vinculin expression

Wound healing assay



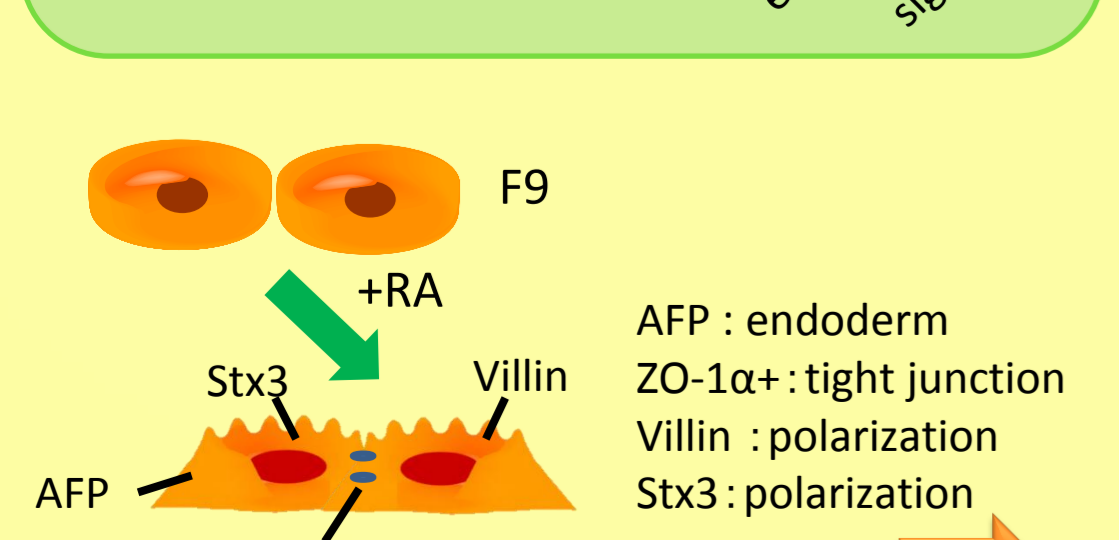
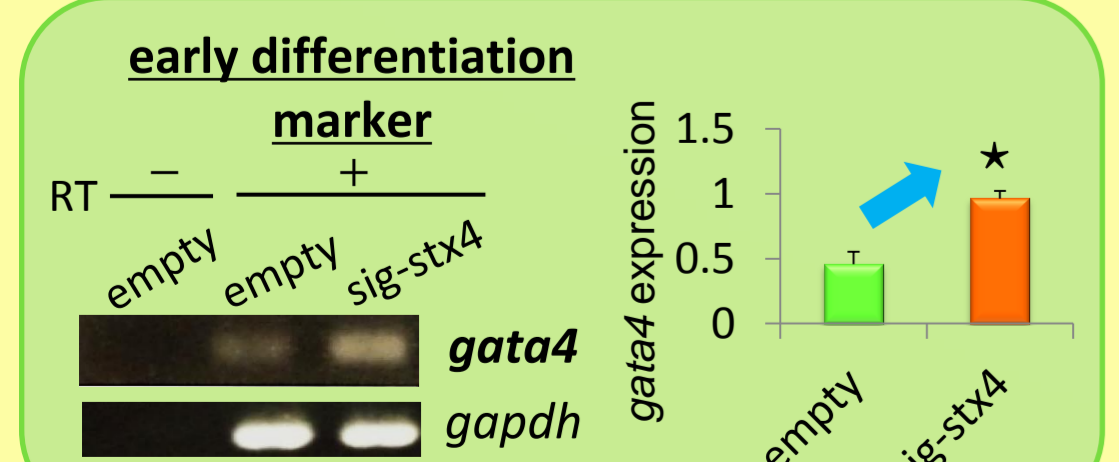
Vinculin



Stx4 → migration ↑
< down-regulation of Vinculin >

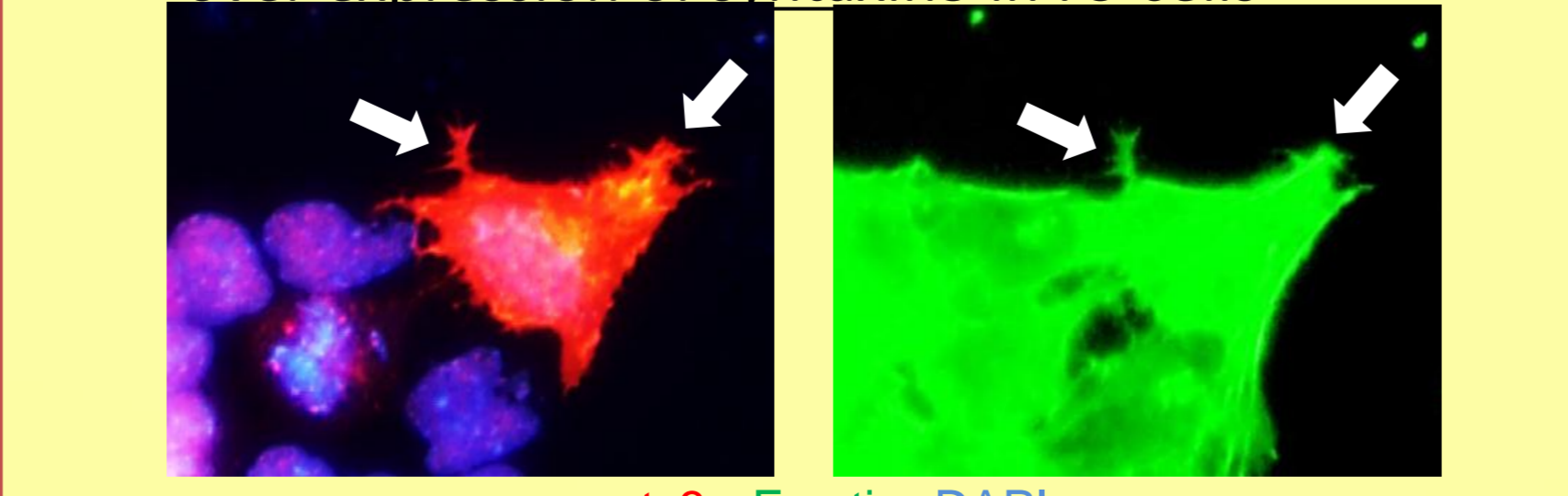
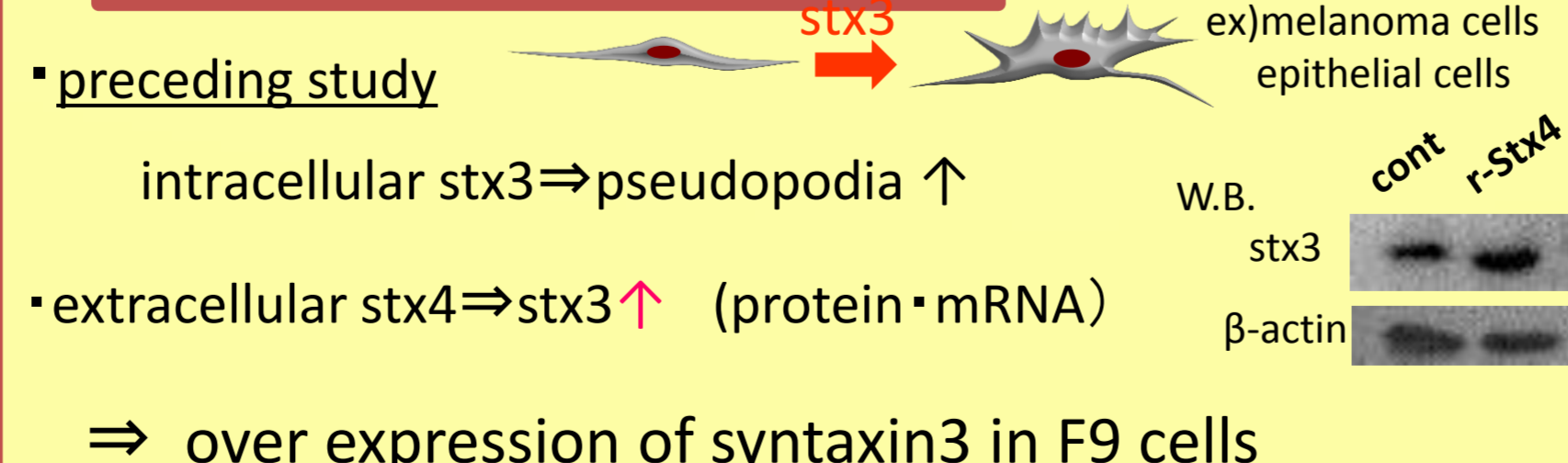
⑥ Effect of stx4 on differentiation

Differentiation markers



stx4 → differentiation ↑
< weaker than RA-treatment, though >

⑦ involvement of stx3



extracellular stx4 → intracellular stx3 ↑
stx3 → pseudopodia ↑, spread
< stx3 partly mediates stx4's extracellular function >

Conclusion

